Methods: Human osteoarthritic chondrocytes were culture with or without Interleukin-1B in the presence or absence of Diacerhein or Rhein for 24 hours. Nitric oxide was estimated via Nitrite quantification by the Griess reagent and apoptosis was investigated by using the Vibrant Apoptosis kit (Molecular Probes). The localisation of FoxO1, FoxO3a, FoxO4 and protein p65 (a member of the nuclear factor-kappaB family) was investigated by fluorescence confocal microscopy. FoxO1, FoxO3a and FoxO4 expression was determinated by Western blotting.
Results: Diacerhein and Rhein reduced IL-1B - induced NO increase and p65 nuclear localisation as previously shown. In control cells, FoxO transcription factors were localisated in the cytoplasm. In the presence of Diacerhein or Rhein, FoxO transcription factors were found to have nuclear localisation whereas they are in the cytoplasm in the presence of IL-1B. When Diacerhein or Rhein are added to the culture medium containing IL-1B, the localisation of FoxO transcription factors changes and become nuclear. No significant changes were observed on FoxO proteins expression among the samples of the study.
Conclusions: Previous studies have shown that Diacerein and its active metabolite reduce the IL-1β deleterious effects on osteoarthritis (OA) cartilage through inhibition of the expression of degrading enzymes. This study presents evidence of the involvement of FoxO transcription factors on diacerein mode of action. It could explain the downregulated proliferation and the increased p27 expression observed on human osteoarthritic chondrocytes in the presence of Rhein recently found by other groups.

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THE ROLE OF THE SWELLING-ACTIVATED Cl^- CURRENT ($I_{CL,SWELL}$) IN THE PROCESS OF REGULATORY VOLUME DECREASE (RVD) IN ISOLATED RABBIT ARTICULAR CHONDROCYTES

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Purpose: Articular chondrocytes are exposed *in vivo* to the continually changing osmotic environment and thus require volume regulatory mechanisms. The present study was designed to investigate i) the functional role of the swelling-activated Cl^- current ($I_{Cl,swell}$) in the regulatory volume decrease (RVD) and ii) the regulatory role of tyrosine phosphorylation in $I_{Cl,swell}$, in isolated rabbit articular chondrocytes.
Methods: Rabbit cartilages were collected from bilateral knee, hip and glenohumeral joints of male animals weighing 2.0 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1-3 days. On the day of experiments, chondrocytes were isolated by enzymatic digestion. Whole-cell membrane current was recorded during exposure to isosmotic (300 mOsm) and hyposmotic (210 mOsm) external solutions under conditions where Na^+, K^+ and Ca^{2+} currents were minimized. To measure of cell volume, isolated chondrocytes were allowed to settle onto the experimental chamber mounted on an inverted microscope. The chamber was continuously perfused with bathing solutions at the rate of 2-3 ml min^{-1}. Microscope images of chondrocytes were recorded with a CCD digital camera equipped with DS-L2 control unit at 2560×1592 resolution every 1 min, and the area of the cell image was measured using Image-J public domain software.
Results: The isolated chondrocytes exhibited a RVD during sustained exposure to hyposmotic solution, which was mostly inhibited by the $I_{Cl,swell}$ blocker DCPIB (4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on-5-yl) oxobutyric acid) at 20 μM. Exposure to a hyposmotic solution activated $I_{Cl,swell}$, which was also largely inhibited by 20 μM DCPIB. Activation of $I_{Cl,swell}$ was significantly reduced by the protein tyrosine kinase (PTK) inhibitor genistein (30 μM) but was scarcely affected by its inactive analogue daidzein (30 μM). Intracellular application of protein tyrosine phosphatase (PTP) inhibitor sodium orthovanadate (250 and 500 μM) resulted in a gradual activation of a Cl^- current even in isosmotic solutions. This Cl^- current was almost completely inhibited by DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid, 500 μM) and was also largely suppressed by exposure to hyperosmotic solution, thus indicating a close similarity to $I_{Cl,swell}$. Pretreatment of chondrocytes with genistein significantly prevented the activation of the Cl^- current by sodium orthovanadate, suggesting that the basal activity of endogenous PTK is required for the activation of this Cl^- current.
Conclusions: Our results provide evidence to indicate that activation of $I_{Cl,swell}$ is involved in RVD and is facilitated by tyrosine phosphorylation in isolated rabbit chondrocytes.

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NON-INVASIVE TREATMENT OF OSTEOARTHRITIS OF THE KNEE WITH QUANTUM MAGNETIC RESONANCE

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Objectives: To study the effects of Quantum Magnetic Resonance (QMR) beams on clinical and functional parameters and cartilage thickness of osteoarthritic knee joints.
Background of QMR Therapy: Quantum Magnetic Resonance Therapy™ utilizes highly complex quantum electromagnetic beams in the sub-radio and near-radio frequency spectrum. The beams can be precisely controlled and focused onto tissues therein generating streaming voltage potentials. In osteoarthritis, this flow in the joint causes forced movement of hydrogen protons in the extra cellular matrix (ECM) due to the alteration in QMR spin in the hydrogen atoms and stimulates the chondrocytes.
Methods: After the publication (2004) of the results of a pilot study on the effect of QMR on 35 patients with osteoarthritis, 300 more patients with osteoarthritis have been treated with QMR as a follow up study. The patients were assessed on the basis of well-established internationally recognized knee society rating system and scores prior to immediately after treatment and further after three months were computed. In addition, MRI of the knees was done using standard protocol before and after three months of treatment with a view to measure the changes in the cartilage thickness in the knee joints.
Results: By the end of the treatment the patients could walk up to five times more than before treatment without any difficulty. MRI showed a remarkable increase in the thickness of the cartilage in the knee joint at three months, from 0.67mm (±0.02) pre-treatment to 3.25mm (±0.74) in left knee, and 0.66mm (±0.02) to 2.71mm (±0.58) in the right knee joint ($p<0.001$).
Conclusions: QMR Therapy™ has now been successfully employed to induce mitotic activity in the fully differentiated chondrocytes. It is also seen that therapeutic exposure to quantum magnetic resonance beams is effective in ameliorating the signs and symptoms of OA, and inducing regenerative activity in the chondrocytes as evidenced by an increase in the cartilage thickness. QMR Therapy™ is a method for regeneration of cartilage and is effective for treatment of osteoarthritis of the knee joint.